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Determination of Boron in Grape (*Vitis vinifera*) by Azomethine H Spectrophotometric Method

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Abstract

Boron content of 10 grape (*Vitis vinifera)* varieties was determined by using Azomethine H spectrophotometric method. The grape samples have been taken from Adana and Niğde region of Turkey. The results varied between 0.59 and 9.51 $mg.kg^{-1}$. Consequently, the differences have been found in boron content of five regions' (Kamışlı, Armutlubağ, Bozyer, Bağaltı, Çatılıyer) grape varieties. The highest boron content was in Armutlubağ region. Results revealed that the Turkish grape is a good natural source of boron.

Keywords: Azomethine H; Grape; Vitis vinifera, Determination of boron

1. Introduction

Boron is one of the trace elements in nature, occurring only in minute concentrations in natural systems. Yet, it is one of the most important trace elements for micronutrients of growing plants [9]. Boron is necessary for vascular plants, diatoms and some species of marine algal flagellates although it is apparently not required by bacteria, fungi, green algae or animals [5].

Boron deficiency in plant may result in reduced growth yield loss, and even death, depending on the severity of deficiency. However, excess boron is toxic to plants [10].

Even though grape include boron is known, there are no data available for boron content of Turkish grapes. In a study, the boron content of red grape is determined as 0.50 mg boron in a 10^5mg sample [4].

Because boron in plants is dependent on the availability of boron in the soil, the same food crop can vary greatly in boron content depending on where it is grown [2]. Boron in food, sodium borate and boric acid are well absorbed from the digestive tract. It has been proposed that

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boron contributes to living systems by acting in directly as a proton donor and that it exerts an influence on cell membrane structure and function [2] and is involved in enzymatic reactions. Boron functions closely with calcium and vitamin D in the preservation of bone mass and the prevention of bone demineralization and stimulates immune system and inflammatory and hormonal responses [8]. The optimal dose of boron for prevention of osteoporosis, osteoarthritis and proper physiological function appears to be between 3-6 $mg.day^{-1}$ [2]. Fruits, legumes, nuts, sea vegetables and vegetables are the most important dietary sources of boron [6].

Thus the determination of boron in plants is becoming increasingly important. In literatures a number of methods have been reported to determination of boron [10] such as spectrophotometry, potentiometry, chromatography, flame atomic emission and absorption spectrophotometry, ICP-OES, ICP-MS, mass spectrometry (MS) and neutron activation analysis [7]. Among all these methods, colorimetric and ICP-OES methods have been extensively applied B determination in plant samples. Colorimetric methods, in general, suffer from numerous interferences and have poor sensitivity and precision. In ICP-OES, besides matrix interferences, the two most sensitive emission lines for B suffer strong spectral interference from Fe. The ICP-OES is not adequately sensitive for some nutritional work involving low B concentrations [7].

ICP-OES and spectrophotometric Azomethine H method methods have been applied for determination of boron in hazelnut. The comparison of the methods indicated that there was no significant difference at a confidence level of 95%. The values found by spectrophotometry and ICP-OES indicating that both methods provide sufficiently accurate results. [8].

There are a number of specific reagents for colorimetric determination of boron. Examples of these reagent are curcumin, carminic acid, quinalizarin, crystal violet, sorbitol, methyl orange, robocurcumin and rosocyanin and methylene blue [11].

In this work, azomethine H spectrophotometric method was used for determination of boron in grape with seed.

The azomethine H method worked in this system is a good method with high sensitivity. Furthermore, it has been purposed that boron content of the same plant in different regions is compared in this study.

2. Materials and Methods

2.1. Materials

Ten grape (*Vitis vinifera*) varieties used in this study, were obtained from Kamışlı, Armutlubağ, Bozyer, Bağaltı, Çatılıyer regions of Turkey at harvest of October 2004. Total sample quantity for all varieties was 10^6 mg each and 10^5mg was taken from this batch. The samples were grinded with their seeds using a blander.

All chemicals used were of analytical grade unless otherwise stated. The aqueous reagents were prepared in distilled water. To avoid possible contamination due to contact with borosilicate glass, all reagent solutions are prepared using plastic ware. Boric acid standard solution (1000 mg L^{-1}) was used for standardization.

2.2. Methods

Three grams of blend grape were weighed in crucibles. Due to the high volatility of boric acid, pH was increased up to approximately 7.0 using 0.1M NaOH. Samples were dried for 12 h at 75°C in oven and the dry samples were kept in furnace for 3 h at 525°C until ashed. Ashes were extracted with 10 mL of 2M HNO_3 and were heated on a hot plate. After dissolution, the contents were filtered by filter paper and diluted to a final volume of 50 mL with distilled water. This final solution was used for analysis. All analysis was made in triplicate.

2.2.1. Spectrophotometric method

The method used for the spectrophotometric determination of micro amounts of boron is based on the boron-catalyzed condensation of salicylaldehyde with H acid. Under the optimum conditions, the wavelength of maximum absorbtion of the yellow complex was found as 412 nm. Boron concentrations were measured in 1.00 cm sample cells, at 412 nm, according to Azomethine H method [3].

2.2.2. Instruments

A Shimadzu double beam UV-VIS spectrophotometer, Model UV-2101 PC, was used for azomethine H method and measurements of pH were made with an Inolab pH meter.

2.2.3. Statistical methods

For the statistical analysis we choose the analysis of variance (ANOVA) in Statistical Analysis System (SPSS 11.0 for windows). The significance of differences between mean values was determined by a multiple range test (LSD; Least Significant Difference). For this reason alpha (α) was preferred to be 0.05 which corresponds to a confidence of level of 95%.

3. Results and discussion

The absorption spectra of azomethine H are measured against the reagent blank (Fig.1.). As the maximum absorption of azomethine H is found at 412 nm, the wavelength of 412 nm was chosen for the spectrophotometric determination of boron in the samples.

Previously reported azomethine H methods have measured the developed color at pH values ranging from 7.5 and 7.8 [3]. Within this pH range, boron-salicylaldehyde chelate takes form. Then the condensation is completed within 15 min at 25 °C and 2.2 pH value. However in the absence of boron and at alkaline pH, the salicylaldehyde ion, itself, exhibits appreciable absorption at approximately 420 nm (Fig.2.). Adjustment of the blank pH between 2.0 and 2.4 for color measurement avoids this interference.

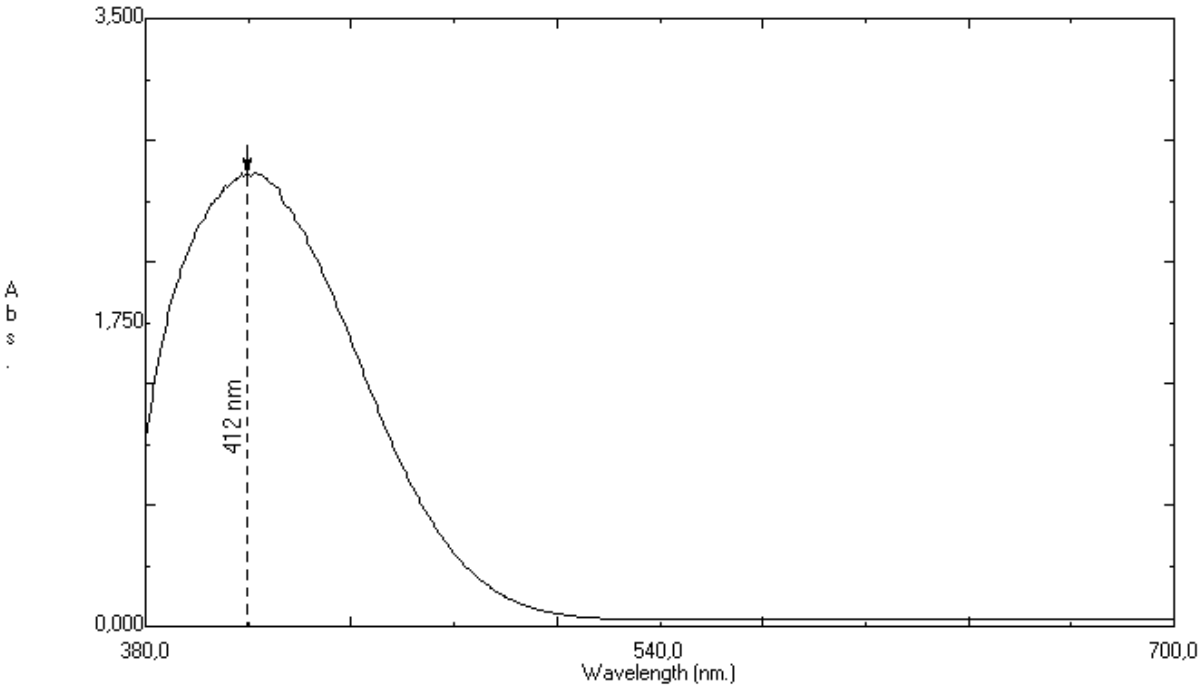

Fig 1. Absorption spectrum of condensation product (Azomethin H) in the presence of 1 mg/L boron, corrected for reagent blank, 1 cm cells.

The tolerance for Ca, Mg and Fe which are present in great amounts in plants may interfere when boron is determined directly in some samples. We found that ascorbic acid EDTA addition to the reaction system can be very effective in masking these ions and greatly improve the selectivity. The amount of EDTA in the buffer masking reagent (2%) increased to extend the tolerance levels for these ions. In this study, azomethine H spectrophotometric method has been applied for determination of boron in white and red varieties of five different regions. Boron contents of samples in these regions have been compared by using SPSS computer program. A comparison of results is given in Table 1, Fig 3 and Fig 4. The table shows that some grape varieties have rather rich boron content and therefore can be used as nutritional source for boron. Both red and white grape varieties regarding boron content, the richest and poorest species are at Armutlubağ region and Bağaltı region, respectively.

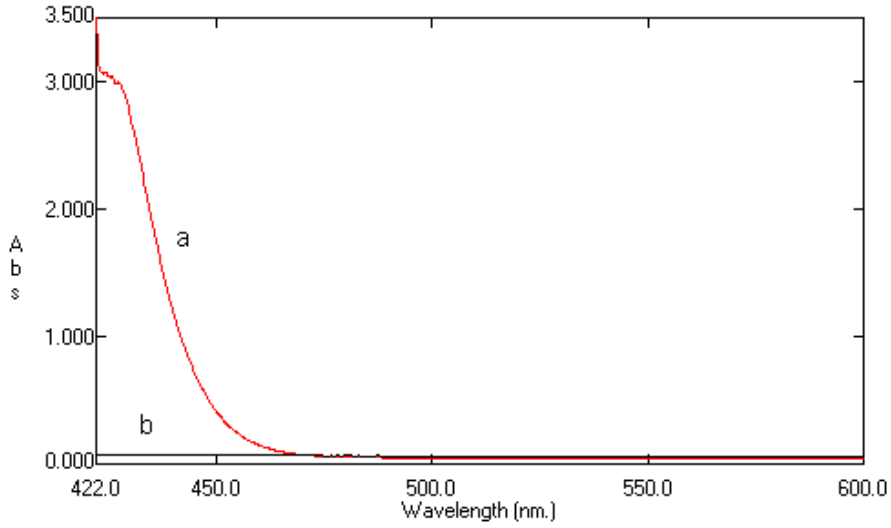

Fig 2. Absorption spectra of 0.1 M salicylaldehyde solutions at pH 7.6 a, and pH 2.2 b.

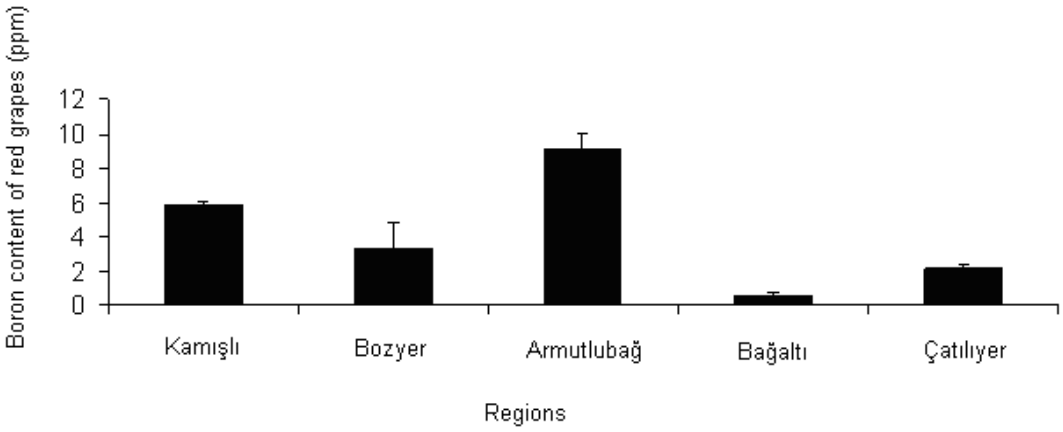

Fig 3. Boron content of red grapes in different regions.

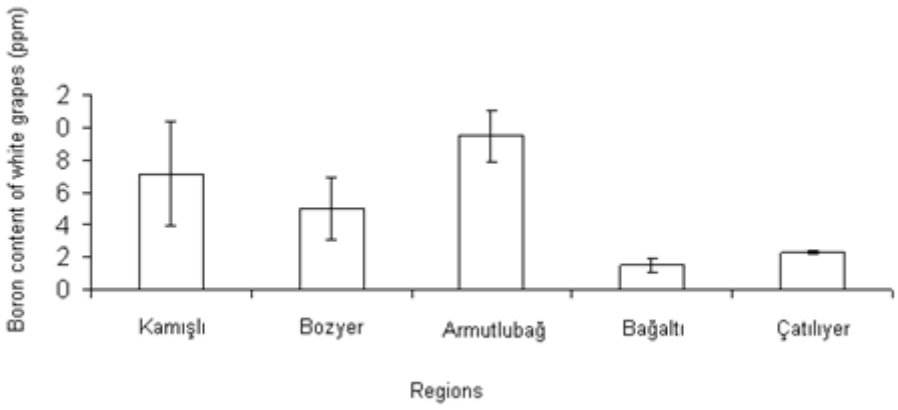

Fig 4. Boron content of white grapes in different regions.

On the other hand, from nutritional point of view, it is known that the hard-crusted fruits are the most important boron sources (~12 mg kg^{-1}); these are followed by fruits (~ 4 $mg.kg^{-1}$) and then vegetables (~ 1.7 mg kg^{-1}) to complete the sequence [1].

The results found in this study that the maximum level for boron content is 9 $mg.kg^{-1}$ (Armutlubağ region) and this is followed by 7 $mg.kg^{-1}$ (Kamışlı region) and then 5 $mg.kg^{-1}$ (Bozyer region). Therefore these regions' grape is rich enough and important source for boron in human nutrition.

Table 1: Boron content of some grapes

	Region	*n*	Boron content ($mg.kg^{-1}$ DW)		
			Mean	S.E. of means	*
Red grape	Kamışlı	3	5.87	0.13	a
	Bozyer	3	3.30	0.87	b
	Armutlubağ	3	9.09	0.54	c
	Bağaltı	3	0.59	0.10	d
	Çatılıyer	3	2.18	0.12	bd
White grape	Kamışlı	3	7.12	1.86	ab
	Bozyer	3	5.02	1.12	a
	Armutlubağ	3	9.51	0.91	b
	Bağaltı	3	1.48	0.27	c
	Çatılıyer	3	2.27	0.05	c

* Means with different letters are significantly different one another according to LSD test ($P<0.05$).

Consequently, any differences have not been found between boron content of red grape and white grape varieties, however the differences have been found in boron content of five regions' grape varieties.

4. Conclusion

Spectrophotometric azomethine H method has adequate sensitivity and accuracy for boron determination in grapes. Azomethine H method is the most common and powerful technique for boron determination. UV-VIS spectrophotometers are easily available in most laboratories and can be confidently and economically used for boron determination in grapes.

The results show that the boron content of grapes in different regions in the same area of Turkey, have been found.

Acknowledgments

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